



# **Rapid qualitative and quantitative analysis of novel drug analogues via Desorption Electrospray Ionisation - Mass Spectrometry (DESI-MS)**

By: Natasha Stojanovska

A thesis submitted for the  
Degree of Doctor of Philosophy (Science),

Centre for Forensic Science,  
University of Technology, Sydney,

2013

*“Learn from yesterday, live for today, hope for tomorrow. The important thing is not to stop questioning.” - Albert Einstein.*

*“To be yourself in a world that is constantly trying to make you something else is the greatest accomplishment.” - Ralph Waldo Emerson.*

## **Certificate of authorship and originality**

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all the information sources and literature used are indicated in the thesis.

NAME: Natasha Stojanovska

DATE:

## Acknowledgements

I would like to express my deepest gratitude to my supervisors Dr. Shanlin Fu, Dr. Mark Tahtouh and Dr. Tamsin Kelly for the continual support and effort throughout the project. I could not have asked for a better group of people to be my mentors.

Dr. Shanlin Fu, I admire your patience, your wisdom and your positive attitude. You have been very supportive along the entire journey and I thank you very much for that. I hope you continue to be the great supervisor that you are and I wish you all the best in your future research endeavours.

Dr. Mark Tahtouh, thank you for your insight, guidance and pure intelligence. You have been a fundamental part of this research and I appreciate all the thought and effort you put into the project.

Dr. Tamsin Kelly, thank you for all your mass spec expertise. Your insight has been invaluable and you have assisted greatly in all aspects of the project. Thank you for putting in the effort to come up from Canberra for meetings and presentations. Your commitment has been second to none and I thank you for that.

I would like to thank Dr. Alison Beavis for assisting greatly in the editing process and for providing thoughtful insight into issues at hand. Thank you for all the DESI/LC/QTOF-MS training. You have been an essential part of this project.

I would like to thank Dr. Ronald Shimmon for all his invaluable assistance in organic synthesis. Thank you to Dr. David Bishop for your technical assistance with the GC-MS and especially the QTOF-MS which proved to be a challenging instrument over the years.

I would like to acknowledge the Australian Federal Police (AFP) for funding and for the samples that were provided for this project. In addition, I acknowledge the AFP for providing the Prosolia™ DESI source for use at the University of Technology, Sydney, for the duration of this project. I would also like to thank Agilent Technologies® for their technical support with the instruments.

I would also like to acknowledge Rochelle Seneviratne from the Science Faculty for all her administrative assistance and for answering all our questions no matter how trivial they

were. I would like to thank everyone in the Centre for Forensic Science for their support and interest in the project and the University of Technology, Sydney, for providing me the opportunity to do my PhD.

I would like to thank my fellow PhD and post-doctoral students: Susan Luong, Anna Molnar, Scott Chadwick, Michael Wood, Marie Morelato, Aimee Lloyd, Joyce Chan, Adrian De Grazia, Regina Verena Taudte, Katelynn Perrault, Maiken Ueland, Kate Grimwood, Unni Kuzhiumparambil and Nathan Charlton and all the other students I had contact with during the years for all the fun times and the amazing support along the way. Finally, a very special thank you goes out to my family and my husband for putting up with me through the hard times and for the unconditional love.

# Table of contents

Certificate of authorship and originality.....	iii
Acknowledgements.....	iv
List of figures.....	xiii
List of tables.....	xxx
Abbreviations.....	xxxii
Abstract.....	xxxvi
<b>CHAPTER 1: INTRODUCTION.....</b>	<b>2</b>
1.1 PREVALENT ILLICIT DRUGS AND NOVEL ANALOGUES.....	2
1.1.1 Amphetamine.....	4
1.1.2 Methylamphetamine.....	5
1.1.3 3,4-Methylenedioxymethylamphetamine.....	5
1.1.4 3,4-Methylenedioxyamphetamine.....	6
1.1.5 N,N-Dimethylamphetamine.....	6
1.1.6 4-Methoxyamphetamine.....	7
1.1.7 Cocaine.....	7
1.1.8 Drug analogues.....	8
1.1.8.1 Piperazine analogues.....	8
1.1.8.2 Cathinone analogues.....	9
1.2 DRUG INTELLIGENCE/PROFILING.....	11
1.3 DRUG ANALYSIS TECHNIQUES.....	14
1.3.1 Current preliminary identification techniques.....	14
1.3.2 Current confirmatory analysis techniques.....	14
1.3.3 Quantification of drug compounds.....	15
1.3.4 Atmospheric pressure ionisation techniques.....	16
1.4 DATABASE AND LIBRARY COMPOUND MATCHING.....	18

1.5	DESORPTION ELECTROSPRAY IONISATION – MASS SPECTROMETRY.....	18
1.5.1	<i>Mechanisms involved in DESI-MS</i> .....	20
1.5.2	<i>Important parameters</i> .....	21
1.5.3	<i>Applications of DESI-MS</i> .....	24
1.5.4	<i>Time-of-Flight - Mass Spectrometry</i> .....	28
1.6	EXPERIMENTAL DESIGN .....	29
1.6.1	<i>Types of experimental design</i> .....	30
1.6.1.1	Full-factorial design.....	30
1.6.1.2	Central composite design .....	31
1.7	AIMS.....	32
<b>CHAPTER 2:</b>	<b>SYNTHESIS.....</b>	<b>34</b>
2.1	INTRODUCTION .....	34
2.2	MATERIALS AND METHODS .....	34
2.2.1	<i>4-Methylmethcathinone (M1 – M4)</i> .....	36
2.2.2	<i>1-Benzylpiperazine (BZP 1 - BZP 4)</i> .....	39
2.2.3	<i>3-Trifluoromethylphenylpiperazine (TFMPP 1 – TFMPP 4)</i> .....	41
2.2.4	<i>3-Chlorophenylpiperazine (mCPP 1)</i> .....	43
2.2.5	<i>4-Methoxyphenylpiperazine (MeOPP 1)</i> .....	44
2.3	RESULTS AND DISCUSSION .....	45
2.3.1	<i>4-Methylmethcathinone</i> .....	45
2.3.1.1	Yield of 4-methylmethcathinone .....	45
2.3.1.2	Purity of 4-methylmethcathinone .....	45
2.3.1.3	2-Chloro-4-methylpropiophenone as intermediate to 4-MMC .....	47
2.3.1.4	2-Bromo-4-methylpropiophenone as intermediate to 4-MMC .....	49
2.3.2	<i>1-Benzylpiperazine</i> .....	51
2.3.3	<i>3-Trifluoromethylphenylpiperazine</i> .....	61
2.3.4	<i>3-Chlorophenylpiperazine</i> .....	68

2.3.5	4-Methoxyphenylpiperazine .....	71
2.4	CONCLUSIONS.....	75
<b>CHAPTER 3: METHOD DEVELOPMENT AND VALIDATION.....</b>		<b>77</b>
3.1	INTRODUCTION .....	77
3.2	MATERIALS AND METHODS .....	78
3.2.1	Sample preparation.....	79
3.2.2	Experimental design .....	79
3.2.2.1	Experimental space.....	80
3.2.2.2	Factor levels.....	81
3.2.2.3	Data analysis .....	81
3.3	RESULTS AND DISCUSSION .....	82
3.3.1	Optimising DESI-MS parameters .....	82
3.3.1.1	Experimental design .....	85
3.3.1.2	One-factor-at-a-time optimisation .....	91
3.3.1.3	Sampling method.....	96
3.3.2	Pharmaceuticals analysis .....	102
3.3.3	Optimising GC-MS parameters.....	103
3.3.3.1	GC-MS conditions .....	104
3.3.3.2	Temperature program .....	104
3.3.4	Optimising LC-MS parameters.....	109
3.3.4.1	LC-MS conditions .....	109
3.3.4.2	Mobile phase gradient development.....	110
3.4	CONCLUSIONS.....	112
<b>CHAPTER 4: ANALYSIS OF AMPHETAMINE-TYPE SUBSTANCES .....</b>		<b>114</b>
4.1	INTRODUCTION .....	114
4.2	MATERIALS AND METHODS .....	114
4.3	RESULTS AND DISCUSSION .....	115



4.3.1	<i>Adulteration and LOD</i> .....	115
4.3.1.1	Methylamphetamine .....	115
4.3.1.2	4-Methoxyamphetamine .....	119
4.3.1.3	Amphetamine .....	120
4.3.1.4	N,N-Dimethylamphetamine .....	122
4.3.1.5	3,4-Methylenedioxymethylamphetamine .....	124
4.3.1.6	Limit of Detection summary .....	127
4.3.2	<i>Analysis of 3,4-methylenedioxymethylamphetamine</i> .....	127
4.3.2.1	Desorption electrospray ionisation – mass spectrometry .....	127
4.3.2.2	Gas chromatography – mass spectrometry .....	133
4.3.2.3	Liquid chromatography – mass spectrometry .....	137
4.3.3	<i>Analysis of 4-methoxymethylamphetamine</i> .....	140
4.3.3.1	Desorption electrospray ionisation – mass spectrometry .....	140
4.3.3.2	Gas chromatography – mass spectrometry .....	143
4.3.3.3	Liquid chromatography – mass spectrometry .....	146
4.3.4	<i>Analysis of dimethylamylamine</i> .....	147
4.3.4.1	Desorption electrospray ionisation – mass spectrometry .....	147
4.3.4.2	Gas chromatography – mass spectrometry .....	150
4.3.4.3	Liquid chromatography – mass spectrometry .....	151
4.3.5	<i>Mass accuracy</i> .....	152
4.4	CONCLUSIONS.....	155
<b>CHAPTER 5:</b>	<b>ANALYSIS OF COCAINE</b> .....	<b>157</b>
5.1	INTRODUCTION .....	157
5.2	MATERIALS AND METHODS .....	157
5.3	RESULTS AND DISCUSSION .....	158
5.3.1	<i>Adulteration and LOD</i> .....	158
5.3.2	<i>Analysis of seized cocaine samples</i> .....	163

5.3.2.1	Desorption electrospray ionisation – mass spectrometry .....	163
5.3.2.2	Gas chromatography – mass spectrometry .....	171
5.3.2.3	Liquid chromatography – mass spectrometry .....	178
5.3.3	<i>Mass accuracy</i> .....	184
5.4	CONCLUSIONS.....	187
<b>CHAPTER 6: ANALYSIS OF PIPERAZINE ANALOGUES .....</b>		<b>189</b>
6.1	INTRODUCTION .....	189
6.2	MATERIALS AND METHODS .....	189
6.3	RESULTS AND DISCUSSION .....	190
6.3.1	<i>Adulteration and LOD</i> .....	190
6.3.1.1	1-Benzylpiperazine.....	190
6.3.1.2	3-Trifluoromethylphenylpiperazine .....	193
6.3.1.3	3-Chlorophenylpiperazine .....	194
6.3.1.4	4-Methoxyphenylpiperazine.....	196
6.3.1.5	Piperazine mixtures .....	198
6.3.2	<i>Analysis of 1-benzylpiperazine</i> .....	200
6.3.2.1	Desorption electrospray ionisation – mass spectrometry .....	200
6.3.2.2	Gas chromatography – mass spectrometry .....	205
6.3.2.3	Liquid chromatography – mass spectrometry .....	209
6.3.3	<i>Analysis of 3-trifluoromethylphenylpiperazine</i> .....	212
6.3.3.1	Desorption electrospray ionisation – mass spectrometry .....	212
6.3.3.2	Gas chromatography – mass spectrometry .....	217
6.3.3.3	Liquid chromatography – mass spectrometry .....	220
6.3.4	<i>Analysis of 3-chlorophenylpiperazine</i> .....	222
6.3.4.1	Desorption electrospray ionisation – mass spectrometry .....	222
6.3.4.2	Gas chromatography – mass spectrometry .....	224
6.3.4.3	Liquid chromatography – mass spectrometry .....	226

6.3.5	<i>Analysis of 4-methoxyphenylpiperazine</i> .....	227
6.3.5.1	Desorption electrospray ionisation – mass spectrometry .....	227
6.3.5.2	Gas chromatography – mass spectrometry .....	229
6.3.5.3	Liquid chromatography – mass spectrometry .....	231
6.3.6	<i>Mass accuracy</i> .....	232
6.4	CONCLUSIONS.....	236
<b>CHAPTER 7: ANALYSIS OF CATHINONE ANALOGUES.....</b>		<b>238</b>
7.1	INTRODUCTION .....	238
7.2	MATERIALS AND METHODS .....	238
7.2.1	<i>Selectivity study</i> .....	238
7.2.2	<i>DESI-MS method validation</i> .....	238
7.2.3	<i>GC-MS method validation</i> .....	241
7.2.4	<i>LC-MS method validation</i> .....	242
7.3	RESULTS AND DISCUSSION .....	243
7.3.1	<i>Qualitative analysis</i> .....	243
7.3.1.1	Desorption electrospray ionisation – mass spectrometry .....	243
7.3.1.2	Gas chromatography – mass spectrometry .....	248
7.3.1.3	Liquid chromatography – mass spectrometry .....	250
7.3.2	<i>Selectivity study</i> .....	250
7.3.2.1	4-Methylmethcathinone and methylene.....	256
7.3.2.2	Differentiating compounds based on MS/MS spectra .....	258
7.3.3	<i>Quantitative analysis</i> .....	261
7.3.3.1	Internal standard .....	261
7.3.3.2	Quantification .....	263
7.3.4	<i>Mass accuracy</i> .....	264
7.4	CONCLUSIONS.....	266
<b>CHAPTER 8: COMPARING DESI-MS TO CURRENT DRUG DETECTION/ANALYSIS TECHNIQUES ....</b>		<b>268</b>

8.1	INTRODUCTION .....	268
8.2	MARQUIS REAGENT .....	268
8.3	CONFIRMATORY ANALYSIS TECHNIQUES.....	268
8.4	COMPARING DESI-MS TO PRELIMINARY IDENTIFICATION TECHNIQUES.....	269
8.5	COMPARING DESI-MS TO QUANTITATIVE ANALYSIS TECHNIQUES.....	270
8.5.1	<i>Gas chromatography – mass spectrometry</i> .....	270
8.5.2	<i>Liquid chromatography – mass spectrometry</i> .....	271
8.5.3	<i>Desorption electrospray ionisation - mass spectrometry</i> .....	272
8.6	FALSE POSITIVE AND FALSE NEGATIVE RESULTS .....	275
8.7	CONCLUSIONS.....	276
<b>CHAPTER 9: CONCLUSIONS AND FUTURE WORK .....</b>		<b>278</b>
9.1	CONCLUSIONS.....	278
9.2	FUTURE WORK.....	281
<b>Appendix.....</b>		<b>282</b>
<b>References.....</b>		<b>298</b>

## List of figures

Figure 1-1 Molecular structures of AP, MA, MDMA, MDA, DMA and PMA. ....	4
Figure 1-2 Molecular structure of cocaine.....	7
Figure 1-3 Molecular structures of BZP, TFMPP, mCPP, MeOPP, and FPP. ....	9
Figure 1-4 Molecular structure of cathinone, methcathinone and 4-MMC. ....	10
Figure 1-5 Generic sites for structural variation of cathinone <sup>6</sup> . ....	10
Figure 1-6 DESI source and moving stage used to position the source <sup>72</sup> . ....	19
Figure 1-7 Simulation of the DESI process showing the formation of dozens of microdroplets resulting from a single droplet-thin film collision event <sup>73</sup> . ....	21
Figure 1-8 Schematic showing DESI parameters requiring optimisation <sup>79</sup> . ....	22
Figure 1-9 Dependence of signal intensity on the spray position at various impact angles from normal <sup>78</sup> . ....	23
Figure 1-10 The Agilent QTOF schematic, showing ion source, ion transfer, optics, beam shaping optics, ion pulsar, flight tube, and detector <sup>93</sup> . ....	29
Figure 1-11 The points of a CCD with three input parameters <sup>94</sup> . ....	31
Figure 2-1 Reaction scheme for the synthesis of 4-methylmethcathinone (M1 – M2) via an $\alpha$ -bromination reaction <sup>95</sup> . ....	36
Figure 2-2 Reaction scheme for the synthesis of 4-methylmethcathinone (M3) via an $\alpha$ -bromination reaction using N-bromosuccinimide <sup>102</sup> . ....	37
Figure 2-3 Reaction scheme for the synthesis of 4-methylmethcathinone (M4) via an $\alpha$ -bromination reaction. ....	38
Figure 2-4 Reaction scheme for the synthesis of 1-benzylpiperazine (BZP 1 - BZP 3) <sup>96</sup> . ....	39
Figure 2-5 Reaction scheme for the synthesis of 1-benzylpiperazine (BZP 4) <sup>97</sup> . ....	40

Figure 2-6 Reaction scheme for the synthesis of 3-trifluoromethylphenylpiperazine (TFMPP 1 – TFMPP 3) <sup>96</sup> .....	41
Figure 2-7 Reaction scheme for the synthesis of 3-trifluoromethylphenylpiperazine (TFMPP 4) <sup>104</sup> .....	42
Figure 2-8 Reaction scheme for the synthesis of 3-chlorophenylpiperazine (mCPP 1) <sup>104</sup> .....	43
Figure 2-9 Reaction scheme for the synthesis of 4-methoxyphenylpiperazine (MeOPP 1). ....	44
Figure 2-10 Calibration curve for 4-MMC standard analysed using GC-MS, codeine-D <sub>6</sub> as IS, n=3 (GC method 1).....	45
Figure 2-11 <sup>1</sup> H NMR of 4-MMC (M1) before vacuum drying. ....	46
Figure 2-12 <sup>1</sup> H NMR of 4-MMC (M1) after vacuum drying. ....	47
Figure 2-13 Reaction scheme for the synthesis of 4-methylmethcathinone using sulfuryl chloride.....	48
Figure 2-14 GC-MS chromatogram of extract 1; 4-methylpropiophenone at 8.9 minutes, 2-chloro-4-methylpropiophenone at 10.3 minutes (GC method 1).....	48
Figure 2-15 EI mass spectrum of extract 1; 4-methylpropiophenone (GC method 1). ....	48
Figure 2-16 EI mass spectrum of extract 1; 2-chloro-4-methylpropiophenone (GC method 1). ....	49
Figure 2-17 GC-MS chromatogram of 2-bromo-4-methylpropiophenone (Br-M4) at 11.2 minutes, 4-methylpropiophenone at 9.1 minutes (GC method 1).....	50
Figure 2-18 EI mass spectrum of 2-bromo-4-methylpropiophenone (Br-M4) (GC method 1). ....	51
Figure 2-19 GC-MS chromatogram of BZP 1.HCl at 4.9 minutes, DBZP at 7.7 minutes (GC method 1).....	53
Figure 2-20 EI mass spectrum of BZP 1.HCl (GC method 1).....	53
Figure 2-21 EI mass spectrum of DBZP (GC method 1).....	54

Figure 2-22 $^1\text{H}$ NMR of BZP 1. ....	54
Figure 2-23 Molecular structure of DBZP. ....	55
Figure 2-24 $^{13}\text{C}$ NMR of BZP 1. ....	55
Figure 2-25 $^{13}\text{C}$ DEPT NMR of BZP 1. ....	56
Figure 2-26 $^1\text{H}$ -NMR of BZP 2 showing ratio of DBZP to BZP signals. ....	57
Figure 2-27 GC-MS chromatogram of BZP 3.HCl at 6.1 minutes, DBZP at 8.5 minutes (GC method 2).....	58
Figure 2-28 GC-MS chromatogram of BZP 4.HCl at 5.0 minutes, MBCP at 6.6 minutes, EBCP at 6.8 minutes, DBZP at 7.7 minutes (GC method 1). ....	59
Figure 2-29 EI mass spectrum of MBCP (GC method 1).....	59
Figure 2-30 EI mass spectrum of EBCP (GC method 1). ....	60
Figure 2-31 Proposed reaction scheme for the formation of ethyl 1-benzyl-4-carboxypiperazine and methyl 1-benzyl-4-carboxypiperazine.....	60
Figure 2-32 Reaction mechanism for the formation of 3-trifluoromethylphenylpiperazine (TFMPP), 2-trifluoromethylphenylpiperazine and 4-trifluoromethylphenylpiperazine. ....	62
Figure 2-33 GC-MS chromatogram of TFMPP 1 at 5.1 minutes (GC method 1). ....	63
Figure 2-34 EI mass spectrum of TFMPP (GC method 1). ....	64
Figure 2-35 A: GC-MS chromatogram of TFMPP 2.HCl at 5.7 minutes and 6.2 minutes, 3-chlorobenzotrifluoride at 3.2 minutes, 3-trifluoromethylphenol at 4.0 minutes; B: EI mass spectrum of 3-trifluoromethylphenol (GC method 2). ....	65
Figure 2-36 A: GC-MS chromatogram of TFMPP 3.HCl at 4.5 minutes, 5.0 minutes, 5.3 minutes; piperazine at 2.5 minutes; B: EI mass spectrum of piperazine (GC method 1). ....	66
Figure 2-37 EI mass spectrum of TFMPP at 4.5 minutes (GC method 1). ....	67

Figure 2-38 EI mass spectrum of TFMPP at 5.0 minutes (GC method 1).....	67
Figure 2-39 EI mass spectrum of TFMPP at 5.3 minutes (GC method 1).....	67
Figure 2-40 A: GC-MS chromatogram of TFMPP 4.HCl at 6.2 minutes, 3-(trifluoromethyl)aniline at 3.8 minutes; B: EI mass spectrum of 3-(trifluoromethyl)aniline (GC method 2).....	68
Figure 2-41 A: GC-MS chromatogram of 1,3-dichlorobenzene at 2.3 minutes; B: EI mass spectrum of 1,3-dichlorobenzene (GC method 1).....	69
Figure 2-42 A: GC-MS chromatogram of mCPP 1.HCl at 7.2 minutes, 3-chloroaniline at 4.5 minutes; B: EI mass spectrum of mCPP (GC method 2).....	70
Figure 2-43 EI mass spectrum of 3-chloroaniline (GC method 2).....	71
Figure 2-44 Structure of 2-chloroanisole starting material. ....	72
Figure 2-45 Proposed reaction mechanism for the formation of 2-methoxyphenylpiperazine and 3-methoxyphenylpiperazine. ....	72
Figure 2-46 A: GC-MS chromatogram of MeOPP 1.HCl at 7.1 minutes, bis(2-chloroethyl)amine at 3.5 minutes, 4-anisidine at 4.3 minutes; B: EI mass spectrum of MeOPP (GC method 2). ..	73
Figure 2-47 EI mass spectrum of 4-anisidine (GC method 2).....	74
Figure 2-48 EI mass spectrum of bis(2-chloroethyl)amine (GC method 2). ....	74
Figure 3-1 NoDoz repeat injection in standard trial. ....	83
Figure 3-2 Codral repeat injection in standard trial.....	83
Figure 3-3 Caffeine KBr (100 mg/g) repeat injection in standard trial. ....	84
Figure 3-4 Depletion of morphine signal over time.....	85
Figure 3-5 Main effects plot for 100 mg/g caffeine KBr disc. ....	86
Figure 3-6 Interaction plot for 100 mg/g caffeine KBr disc.....	87



Figure 3-7 Effects plot for 100mg/g caffeine in KBr disc. ....	88
Figure 3-8 Residual plots for 100 mg/g caffeine KBr disc. ....	89
Figure 3-9 Response surface plots for 100 mg/g caffeine KBr disc.....	90
Figure 3-10 Contour plot for 100 mg/g caffeine KBr disc. ....	90
Figure 3-11 Optimisation plot for 100 mg/g caffeine KBr disc. ....	91
Figure 3-12 Optimising solvent flow rate (mL/hr) for 100 mg/g caffeine KBr disc, n=5. ....	93
Figure 3-13 Optimising gas pressure for 100 mg/g caffeine KBr disc, n=5. ....	93
Figure 3-14 Optimising spray high voltage for 100 mg/g caffeine KBr disc, n=5. ....	94
Figure 3-15 Optimising solvent composition for 100 mg/g caffeine KBr disc, n=5.....	95
Figure 3-16 Optimising fragmentor voltage for 100 mg/g caffeine KBr disc, n=5. ....	96
Figure 3-17 Paracetamol tablet being analysed by DESI-MS. ....	97
Figure 3-18 DESI-MS spectra of 4-MMC; A: M1, B: M2, C: M3, D: M4 in KBr disc.....	98
Figure 3-19 DESI-MS spectra of 4-MMC; A: M1, B: M2, C: M3, D: M4 on PVC plate.....	99
Figure 3-20 DESI-MS spectra of 4-MMC; A: M1, B: M2, C: M3, D: M4 powder on PVC plate.	100
Figure 3-21 Double sided tape with sample powders for DESI-MS analysis. 0=blank, 1=M1, 2=M2, 5=M3, 6=M4, C=Caffeine. ....	100
Figure 3-22 DESI-MS spectra of 4-MMC; A: M1, B: M2, C: M3, D: M4 on double sided tape.	101
Figure 3-23 A: Polyvinyl chloride (PVC); B: Polymethyl methacrylate (pMMA); C: Polytetrafluoroethylene (PTFE). ....	101
Figure 3-24 GC-MS chromatogram of codeine-D <sub>6</sub> using gradient A. ....	105
Figure 3-25 GC-MS chromatogram of codeine-D <sub>6</sub> using gradient B. ....	106

Figure 3-26 GC-MS chromatogram of codeine-D <sub>6</sub> using gradient C. ....	106
Figure 3-27 GC-MS chromatogram of codeine-D <sub>6</sub> using gradient D. ....	107
Figure 3-28 GC-MS chromatogram of 4-MMC; 2 µL injection, splitless mode. ....	108
Figure 3-29 GC-MS chromatogram of 4-MMC and codeine-D <sub>6</sub> using gradient D; 2 µL injection, splitless mode. ....	109
Figure 3-30 LC-MS EIC of 4-MMC and codeine-D <sub>6</sub> , gradient 1, resolution = 1.1. ....	111
Figure 3-31 LC-MS EIC of 4-MMC and codeine-D <sub>6</sub> , gradient 2, resolution = 2.3. ....	111
Figure 3-32 LC-MS EIC of 4-MMC and codeine-D <sub>6</sub> , gradient 3, resolution = 2.6. ....	111
Figure 4-1 Chemical structures of common adulterants in ATS preparations. ....	116
Figure 4-2 Intra-day study of the effects of different adulterants on the detection of MA (0.36 µg), 1:1 ratio, n=3. ....	116
Figure 4-3 Inter-day study of the effect of different adulterants on the detection of MA (0.36 µg), 1:1 ratio, n=3. ....	117
Figure 4-4 Adulterating MA standard with caffeine at varying amounts caffeine added (0 %, 20 %, 50 %, 90 %, 95 % w/w), n=3, 2 µL of 74.5 µg/mL, equivalent to 0.14 µg MA. ....	118
Figure 4-5 A: DESI-MS of MA, B: MS/MS of MA at 20 eV. ....	118
Figure 4-6 Proposed collision induced dissociation of the [M+H] <sup>+</sup> ion of MA. ....	118
Figure 4-7 Adulterating PMA standard with caffeine at varying amounts of caffeine added (0 %, 20 %, 50 %, 90 %, 95 % w/w), n=3, 2 µL 826 µg/mL, equivalent to 1.65 µg PMA. ....	119
Figure 4-8 A: DESI-MS of PMA, B: MS/MS of PMA at 20 eV. ....	120
Figure 4-9 Proposed collision induced dissociation of the [M+H] <sup>+</sup> ion of PMA. ....	120
Figure 4-10 Adulterating AP standard with caffeine at varying amounts of caffeine added (0 %, 20 %, 50 %, 90 %, 95 % w/w), n=3, 2 µL of 67.6 µg/mL, equivalent to 0.14 µg AP. ....	121

Figure 4-11 A: DESI-MS of AP, B: MS/MS of AP at 20 eV. ....	121
Figure 4-12 Proposed collision induced dissociation of the $[M+H]^+$ ion of AP. ....	122
Figure 4-13 Inter-day study of AP adulterated with caffeine (0 %, 20 %, 50 %, 90 %, 95 % w/w); n=3, (2 $\mu$ L of 67.6 $\mu$ g/mL, equivalent to 0.14 $\mu$ g AP).....	122
Figure 4-14 Adulterating DMA standard with caffeine at varying amounts of caffeine added (0 %, 20 %, 50 %, 90 %, 95 % w/w), n=3, 2 $\mu$ L of 80 $\mu$ g/mL, equivalent to 0.16 $\mu$ g DMA. ....	123
Figure 4-15 A: DESI-MS of DMA, B: MS/MS of DMA at 20 eV. ....	124
Figure 4-16 Proposed collision induced dissociation of the $[M+H]^+$ ion of DMA. ....	124
Figure 4-17 Adulterating MDMA standard with caffeine at varying amounts of caffeine added (0 %, 20 %, 50 %, 90 %, 95 % w/w), n=3, 2 $\mu$ L of 9660 $\mu$ g/mL, equivalent to 19.4 $\mu$ g MDMA. ....	125
Figure 4-18 A: DESI-MS of MDMA; B: MS/MS of MDMA; C: MS/MS of caffeine at 20 eV. ....	126
Figure 4-19 Proposed collision induced dissociation of the $[M+H]^+$ ion of MDMA. ....	126
Figure 4-20 Data on MDMA sample provided by AFP (analysis conducted by NMI).....	128
Figure 4-21 DESI-MS spectra of MDMA tablet.....	129
Figure 4-22 A: DESI-MS of MDMA tablet, B: MS/MS of MDMA, C: MS/MS of 3,4-methylenedioxyphenyl-2-propanol (MDP-2-POH), D: MS/MS of 3,4-methylenedioxymethylamphetamine (MDDMA). ....	129
Figure 4-23 Proposed collision induced dissociation of the $[M+H]^+$ ion of MDMA (- - >), MDP-2-POH (→) and MDDMA (→). ....	130
Figure 4-24 A: DESI-MS of MDMA sample, B: MS/MS of MDMA, C: MS/MS of MDP-2-P, D: MS/MS of PN.....	131
Figure 4-25 Proposed collision induced dissociation of the $[M+H]^+$ ion of MDP-2-P (- - >) and PN (→). ....	131

Figure 4-26 PCDL library match to MDMA.....	132
Figure 4-27 GC-MS chromatogram of MDMA tablet; MDP-2-POH at 6.1 minutes, MDMA at 6.3 minutes, MDDMA at 6.5 minutes (GC method 2).....	133
Figure 4-28 EI mass spectrum of MDMA (GC method 2).....	134
Figure 4-29 EI mass spectrum of MDP-2-POH (GC method 2).....	134
Figure 4-30 EI mass spectrum of MDDMA (GC method 2). ....	135
Figure 4-31 Proposed EI fragmentation of MDMA tablet.....	135
Figure 4-32 GC-MS chromatogram of synthesised MDMA base; PN at 5.4 minutes, MDMA at 6.2 minutes, MDP-2-P at 6.6 minutes, N-formyl-MDMA at 7.7 minutes (GC method 2). ....	136
Figure 4-33 EI mass spectrum of PN (GC method 2).....	136
Figure 4-34 EI mass spectrum of MDP-2-P (GC method 2). ....	137
Figure 4-35 EI mass spectrum of N-formyl-MDMA (GC method 2). ....	137
Figure 4-36 LC-MS chromatogram of MDMA tablet; A: EIC of MDMA at 4.9 minutes, m/z 194; B: EIC of MDP-2-POH (trace) at 1.3 minutes, m/z 181; C: EIC of MDDMA at 5.1 minutes, m/z 208. ....	138
Figure 4-37 LC-MS spectra of MDMA tablet; A: MDMA, B: MDP-2-POH, C: MDDMA.....	139
Figure 4-38 LC-MS chromatogram of MDMA base; A: EIC of MDMA at 4.8 minutes, m/z 194; B: EIC of MDP-2-P at 1.8 minutes, m/z 179; C: EIC of N-formylMDMA at 10.2 minutes, m/z 222. ....	139
Figure 4-39 LC-MS/MS spectra of MDMA base; A: MDMA; B: MDP-2-P; C: N-formyl-MDMA. ....	140
Figure 4-40 A: DESI-MS spectra of PMMA; B: MS/MS of PMMA.....	141
Figure 4-41 Proposed collision induced dissociation of the $[M+H]^+$ ion of PMMA. ....	141

Figure 4-42 PCDL library match to PMMA. ....	142
Figure 4-43 A: GC-MS chromatogram of synthesised PMMA, anethole at 4.9 minutes, PMMA at 5.8 minutes, PMP-2-P at 6.4 minutes; B: EI mass spectrum of PMMA (GC method 2). ....	144
Figure 4-44 EI mass spectrum of anethole (GC method 2). ....	145
Figure 4-45 EI mass spectrum of PMP-2-P (GC method 2). ....	145
Figure 4-46 Proposed EI fragmentation of PMMA base. ....	146
Figure 4-47 LC-MS chromatogram of PMMA base; A: EIC of PMMA at 10.3 minutes, m/z 180; B: EIC of anethole at 5.1 minutes, m/z 149; C: EIC of PMP-2-P at 19.8 minutes, m/z 165. ....	146
Figure 4-48 LC-MS/MS of PMMA base; A: PMMA; B: anethole; C: PMP-2-P. ....	147
Figure 4-49 A: DESI-MS spectra of "Jack3d" containing DMAA and caffeine, B: MS/MS of DMAA, C: MS/MS of caffeine. ....	148
Figure 4-50 Proposed collision induced dissociation of the $[M+H]^+$ ion of DMAA. ....	148
Figure 4-51 Proposed collision induced dissociation of the $[M+H]^+$ ion of caffeine. ....	148
Figure 4-52 PCDL library match to DMAA. ....	149
Figure 4-53 A: GC-MS chromatogram of "Jack3d", caffeine at 7.5 minutes; B: EI mass spectrum of caffeine (GC method 2). ....	150
Figure 4-54 LC-MS chromatogram of "Jack3d"; A: EIC of caffeine at 4.1 minutes, m/z 195; B: EIC of creatine monohydrate at 1.2 minutes, m/z 132; C: EIC of DMAA at 4.9 minutes, m/z 116. ....	151
Figure 4-55 LC-MS/MS spectra of "Jack3d"; A: caffeine, B: creatine monohydrate, C: DMAA. ....	151
Figure 5-1 Chemical structures of common adulterants in illicit cocaine preparations. ....	159
Figure 5-2 Intra-day study of the effects of different adulterants on the detection of cocaine, n=3. ....	159

Figure 5-3 Inter-day study of the effect of different adulterants on the detection of cocaine, n=3. ....	160
Figure 5-4 Adulterating cocaine standard with varying amounts of caffeine added (i.e. 0 %, 20 %, 50 %, 90 %, 95 % w/w), n=3, 2 µL of 12130 µg/mL, equivalent to 24.3 µg cocaine. ....	161
Figure 5-5 A: DESI-MS spectra of cocaine; B: MS/MS spectra of cocaine at 20 eV. ....	161
Figure 5-6 Proposed collision induced dissociation of the $[M+H]^+$ ion of cocaine <sup>126</sup> . ....	162
Figure 5-7 Data on cocaine samples provided by AFP (analysis conducted by NMI). ....	165
Figure 5-8 DESI-MS spectra of AFP cocaine samples; A: Item 1, B: Item 1/2, C: Item 5/2. ....	166
Figure 5-9 DESI-MS/MS spectra of cocaine item 5/2; A: cocaine, B: CC, C: truxilline, D: hydroxyzine, E: EME, F: levamisole. ....	166
Figure 5-10 Proposed collision induced dissociation of the $[M+H]^+$ ion of CC. ....	167
Figure 5-11 Proposed collision induced dissociation of the $[M+H]^+$ ion of $\alpha$ -truxilline. ....	167
Figure 5-12 Proposed collision induced dissociation of the $[M+H]^+$ ion of hydroxyzine. ....	168
Figure 5-13 Proposed collision induced dissociation of the $[M+H]^+$ ion of EME. ....	168
Figure 5-14 Proposed collision induced dissociation of the $[M+H]^+$ ion of levamisole. ....	168
Figure 5-15 Proposed collision induced dissociation of the $[M+H]^+$ ion of 3,4,5-trimethoxycocaine. ....	169
Figure 5-16 PCDL library match to cocaine. ....	170
Figure 5-17 A: GC-MS chromatogram of cocaine Item 1, benzoic acid at 4.6 minutes, ecgonidine methyl ester at 5.7 minutes, EME at 6.1 minutes, caffeine at 7.6 minutes, tropacocaine at 7.9 minutes, cocaine at 8.8 minutes, 3,4,5-trimethoxycocaine at 9.1 minutes, CC at 9.7 minutes; B: EI mass spectrum of cocaine (GC method 2). ....	171
Figure 5-18 EI mass spectrum of ecgonidine methyl ester (GC method 2). ....	172

Figure 5-19 EI mass spectrum of EME (GC method 2). .....	172
Figure 5-20 EI mass spectrum of caffeine (GC method 2). .....	173
Figure 5-21 EI mass spectrum of tropacocaine (GC method 2). .....	173
Figure 5-22 EI mass spectrum of 3,4,5-trimethoxycocaine (GC method 2). .....	174
Figure 5-23 EI mass spectrum of CC (GC method 2). .....	174
Figure 5-24 GC-MS chromatogram of cocaine standard; benzoic acid methyl ester at 4.2 minutes, ecgonidine methyl ester at 5.7 minutes, EME at 6.1 minutes, cocaine at 8.7 minutes (GC method 2). .....	175
Figure 5-25 GC-MS chromatogram of cocaine Item 1/2; benzoic acid at 4.7 minutes, ecgonidine methyl ester at 5.7 minutes, EME at 6.2 minutes, caffeine at 7.6 minutes, cocaine at 8.8 minutes, CC at 9.4 minutes, BE at 10.1 minutes (GC method 2). .....	175
Figure 5-26 EI mass spectrum of BE (GC method 2). .....	176
Figure 5-27 Chemical structures of benzoic acid, ecgonidine methyl ester and benzoylecgonine. ....	176
Figure 5-28 GC-MS chromatogram of cocaine Item 5/2; benzoic acid at 4.7 minutes, ecgonidine methyl ester at 5.7 minutes, EME at 6.1 minutes, caffeine at 7.6 minutes, levamisole at 8.1 minutes, cocaine at 8.8 minutes, CC at 9.7 minutes, BE at 10.1 minutes (GC method 2). .....	177
Figure 5-29 EI mass spectrum of levamisole (GC method 2). .....	177
Figure 5-30 Proposed EI fragmentation pathway for cocaine. ....	178
Figure 5-31 LC-MS chromatogram of cocaine Item 1; A: EIC of cocaine at 7.2 minutes, m/z 304; B: EIC of truxillines at 9.2 minutes, m/z 659; C: EIC of 3,4,5-trimethoxycocaine at 8.1 minutes, m/z 394; D: EIC of tropacocaine at 6.3 minutes, m/z 246; E: EIC of caffeine at 4.2 minutes, m/z 195; F: EIC of EME at 1.5 minutes, m/z 200; G: EIC of CC at 9.6 minutes, m/z 330. ....	179

Figure 5-32 LC-MS/MS spectra of cocaine Item 1; A: cocaine, B: truxilline, C: 3,4,5-trimethoxycocaine, D: tropacocaine, E: caffeine, F: EME, G: CC at 20 eV. ....	180
Figure 5-33 LC-MS chromatogram of cocaine Item 1/2; A: EIC of cocaine at 7.2 minutes, m/z 304; B: EIC of truxillines at 10.2 minutes, m/z 659; C: EIC of 3,4,5-trimethoxycocaine at 8.1 minutes, m/z 394; D: EIC of caffeine at 4.2 minutes, m/z 195; E: EIC of EME at 1.5 minutes, m/z 200; F: EIC of CC at 9.4 minutes, m/z 330. ....	181
Figure 5-34 LC-MS/MS spectra of cocaine Item 1/2; A: cocaine, B: truxilline, C: 3,4,5-trimethoxycocaine, D: caffeine, E: EME, F: CC at 20 eV. ....	181
Figure 5-35 LC-MS chromatogram of cocaine Item 5/2; A: EIC of cocaine at 7.3 minutes, m/z 304; B: EIC of truxillines at 9.2 minutes, m/z 659; C: EIC of 3,4,5-trimethoxycocaine at 8.1 minutes, m/z 394; D: EIC of levamisole at 5.0 minutes, m/z 205; E: EIC of hydroxyzine at 12.1 minutes, m/z 375; F: EIC of caffeine at 4.2 minutes, m/z 195; G: EIC of EME at 1.5 minutes, m/z 200; H: EIC of CC at 9.6 minutes, m/z 330. ....	182
Figure 5-36 LC-MS/MS spectra of cocaine Item 5/2; A: cocaine, B: truxilline, C: 3,4,5-trimethoxycocaine, D: levamisole, E: hydroxyzine, F: caffeine, G: EME, H: CC at 20 eV. ....	183
Figure 5-37 LC-MS/MS spectra of cocaine standard; A: EME at m/z 200, B: cocaine at m/z 304. ....	183
Figure 6-1 Adulterating BZP 2 with caffeine at varying amounts of caffeine added (0 %, 20 %, 50 %, 90 %, 95 % w/w), n=3, 2 $\mu$ L of 7050 $\mu$ g/mL, equivalent to 14.10 $\mu$ g BZP. ....	191
Figure 6-2 A: DESI-MS spectra of BZP 2; B: MS/MS spectra of BZP at 20 eV. ....	191
Figure 6-3 Proposed collision induced dissociation of the $[M+H]^+$ ion of BZP. ....	192
Figure 6-4 Inter-day study of BZP adulterated with caffeine (0 %, 20 %, 50 %, 90 %, 95 % w/w); n=3, (2 $\mu$ L of 7050 $\mu$ g/mL, equivalent to 14.10 $\mu$ g BZP). ....	192
Figure 6-5 Adulterating TFMPP standard with caffeine at varying amounts of caffeine added (0 %, 20 %, 50 %, 90 %, 95 % w/w), n=3, 2 $\mu$ L of 920 $\mu$ g/mL, equivalent to 1.84 $\mu$ g TFMPP. ....	193
Figure 6-6 A: DESI-MS spectra of TFMPP; B: MS/MS spectra of TFMPP at 20 eV. ....	194



Figure 6-7 Proposed collision induced dissociation of the $[M+H]^+$ ion of TFMPP. ....	194
Figure 6-8 Adulterating mCPP standard with caffeine at varying amounts of caffeine added (0 %, 20 %, 50 %, 90 %, 95 % w/w), n=3, 2 $\mu$ L of 8000 $\mu$ g/mL, equivalent to 16.0 $\mu$ g mCPP.....	195
Figure 6-9 A: DESI-MS spectra of mCPP; B: MS/MS spectra of mCPP at 20 eV. ....	196
Figure 6-10 Proposed collision induced dissociation of the $[M+H]^+$ ion of mCPP. ....	196
Figure 6-11 Adulterating MeOPP 1 with caffeine at varying amounts of caffeine added (0 %, 20 %, 50 %, 90 %, 95 % w/w), n=3, 2 $\mu$ L of 1560 $\mu$ g/mL, equivalent to 3.10 $\mu$ g MeOPP.....	197
Figure 6-12 A: DESI-MS spectra of MeOPP 1; B: MS/MS spectra of MeOPP at 20 eV.....	198
Figure 6-13 Proposed collision induced dissociation of the $[M+H]^+$ ion of MeOPP.....	198
Figure 6-14 Adulterating a mixture of BZP 1 HCl and TFMPP 2 base (1:1) with varying amounts of caffeine, n=3. ....	199
Figure 6-15 DESI-MS spectra of A: BZP 1, B: BZP 2, C: BZP 3. ....	201
Figure 6-16 DESI-MS/MS of A: BZP 1 and B: DBZP.....	201
Figure 6-17 Proposed collision induced dissociation of the $[M+H]^+$ ion of BZP (- - >) and DBZP ( $\rightarrow$ ). ....	202
Figure 6-18 PCDL library match to BZP. ....	203
Figure 6-19 DESI-MS spectra of A: BZP 4, B: MS/MS of BZP, C: MS/MS of DBZP, D: MS/MS of MBCP, E: MS/MS of MS/MS of EBCP, F: MS/MS of benzyl chloride.....	204
Figure 6-20 Proposed collision induced dissociation of the $[M+H]^+$ ion of MBCP (by-product, $\rightarrow$ ), EBCP (intermediate, $\rightarrow$ ) and benzyl chloride (- - >).....	205
Figure 6-21 GC-MS chromatogram of BZP 1.HCl at 6.1 minutes, DBZP at 8.5 minutes (GC method 2).....	206
Figure 6-22 EI mass spectra of BZP 1.HCl (GC method 2). ....	206

Figure 6-23 Proposed EI fragmentation pathway for BZP. ....	207
Figure 6-24 GC-MS chromatogram of BZP 2.HCl at 6.1 minutes, DBZP at 8.5 minutes (GC method 2).....	207
Figure 6-25 GC-MS chromatogram of BZP 3.HCl at 6.1 minutes, DBZP at 8.5 minutes (GC method 2).....	208
Figure 6-26 GC-MS chromatogram of BZP 4.HCl at 5.0 minutes, MDCP at 6.6 minutes, EBCP, 6.8 minutes, DBZP at 7.7 minutes (GC method 1). ....	208
Figure 6-27 LC-MS chromatogram of BZP 1.HCl; A: EIC of BZP at 4.3 minutes, m/z 177; B: EIC of DBZP at 11.3 minutes, m/z 267. ....	209
Figure 6-28 LC-MS chromatogram of BZP 2.HCl; A: EIC of BZP at 4.3 minutes, m/z 177; B: EIC of DBZP at 11.3 minutes, m/z 267. ....	209
Figure 6-29 LC-MS chromatogram of BZP 3.HCl; A: EIC of BZP at 4.3 minutes, m/z 177; B: EIC of DBZP at 11.3 minutes, m/z 267. ....	210
Figure 6-30 LC-MS/MS spectra of A: BZP, B: DBZP. ....	210
Figure 6-31 LC-MS chromatogram of BZP 4.HCl A: EIC of BZP at 4.3 minutes, m/z 177; B: EIC of MBCP at 11.1 minutes, m/z 235; C: EIC of DBZP at 11.8 minutes, m/z 267; D: EIC of EBCP at 13.0 minutes, m/z 249. ....	211
Figure 6-32 LC-MS/MS of A: BZP, B: MBCP, C: DBZP, D: EBCP.....	211
Figure 6-33 DESI-MS spectra of A: TFMPP 1, B: TFMPP 2, C: TFMPP 3, D: TFMPP 4. ....	212
Figure 6-34 DESI-MS/MS of TFMPP 2 and TFMPP 3; A: TFMPP, B: 3-trifluoromethylphenol, C: piperazine, D: 3-(trifluoromethyl)aniline. ....	214
Figure 6-35 PCDL library match to TFMPP.....	215
Figure 6-36 Proposed collision induced dissociation of the $[M+H]^+$ ion of TFMPP, TFMP ( $\rightarrow$ ), 3-(trifluoromethyl)aniline ( $-->$ ) and piperazine. ....	216
Figure 6-37 GC-MS chromatogram of TFMPP 1.HCl at 5.1 minutes (GC method 1).....	217

Figure 6-38 EI mass spectra for TFMPP 1 (GC method 1). .....	218
Figure 6-39 Proposed EI fragmentation pathway for TFMPP. ....	218
Figure 6-40 GC-MS chromatogram of TFMPP 2.HCl at 5.7 minutes and 6.2 minutes, 3-chlorobenzotrifluoride at 3.3 minutes, 3-trifluoromethylphenol at 4.0 minutes (GC method 2). .....	219
Figure 6-41 GC-MS chromatogram of TFMPP 3.HCl at 4.5 minutes, 5.0 minutes, 5.3 minutes; piperazine at 2.5 minutes (GC method 1). ....	219
Figure 6-42 GC-MS chromatogram of TFMPP 4.HCl at 6.2 minutes, 3-(trifluoromethyl)aniline at 3.8 minutes (GC method 2). ....	220
Figure 6-43 LC-MS chromatogram of TFMPP; A: EIC of TFMPP 1 at 8.0 minutes, m/z 231; B: EIC of TFMPP 2; C: EIC of TFMPP 3; D: EIC of TFMPP 4; E: EIC of 3-(trifluoromethyl)aniline at 3.9 minutes, m/z 162; F: MS/MS of TFMPP. ....	221
Figure 6-44 DESI-MS spectra of A: mCPP 1, B: MS/MS spectra of mCPP, C: MS/MS spectra of 3-chloroaniline. ....	222
Figure 6-45 PCDL library match to mCPP. ....	223
Figure 6-46 Proposed collision induced dissociation of the $[M+H]^+$ ion of mCPP and 3-chloroaniline. ....	224
Figure 6-47 GC-MS chromatogram of mCPP 1.HCl at 7.2 minutes, 3-chloroaniline at 4.5 minutes (GC method 2). ....	225
Figure 6-48 EI mass spectrum of mCPP 1 (GC method 2). ....	225
Figure 6-49 Proposed EI fragmentation pathway for mCPP. ....	226
Figure 6-50 LC-MS chromatogram of mCPP 1; A: EIC of mCPP at 6.4 minutes, m/z 197; B: MS/MS of mCPP. ....	226
Figure 6-51 DESI-MS spectra of A: MeOPP 1, B: MS/MS spectra of MeOPP, C: MS/MS spectra of 4-anisidine. ....	227

Figure 6-52 PCDL library match to MeOPP. ....	228
Figure 6-53 Proposed collision induced dissociation of the $[M+H]^+$ ion of MeOPP and 4-anisidine. ....	229
Figure 6-54 GC-MS chromatogram of MeOPP 1.HCl at 7.2 minutes, bis(2-chloroethyl)amine at 3.5 minutes, 4-anisidine at 4.3 minutes (GC method 2). ....	230
Figure 6-55 EI mass spectra of MeOPP 1 (GC method 2). ....	230
Figure 6-56 Proposed EI fragmentation pathway for MeOPP. ....	231
Figure 6-57 LC-MS chromatogram of MeOPP; A: EIC of MeOPP 1 at 4.2 minutes, m/z 193; B: EIC of 4-anisidine at 5.8 minutes, m/z 124; C: MS/MS of MeOPP 1 ....	232
Figure 7-1 DESI-MS calibration curve for 4-MMC, n=3. ....	239
Figure 7-2 GC-MS calibration curve for 4-MMC, n=3 (GC method 1). ....	241
Figure 7-3 LC-MS calibration curve for 4-MMC, n=3. ....	242
Figure 7-4 DESI-MS of 4-MMC (M1, M2, M3 and M4) in positive ion mode. ....	244
Figure 7-5 A: MS of 4-MMC, B: MS/MS spectra of triethylamine, C: MS/MS spectra of 4-MMC product ion at m/z 178 in positive ion mode at 30 eV. ....	245
Figure 7-6 Proposed collision induced dissociation of the $[M+H]^+$ ion of 4-MMC. ....	246
Figure 7-7 Proposed collision induced dissociation of the $[M+H]^+$ ion of triethylamine. ....	246
Figure 7-8 PCDL library match to 4-MMC. ....	247
Figure 7-9 GC-MS chromatogram of 4-MMC (M4) at 4.4 minutes, 2-methylpropiophenone at 3.6 minutes, 2-bromo-4-methylpropiophenone at 4.1 minutes (GC method 1). ....	248
Figure 7-10 EI mass spectra of 4-MMC (GC method 1). ....	249
Figure 7-11 Proposed EI fragmentation pathway of 4-MMC. ....	249

Figure 7-12 LC-MS chromatogram of 4-MMC A: EIC of M1 at 5.7 minutes, m/z 178; B: EIC of M2; C: EIC of M3; D: EIC of M4; E: LC-MS/MS of 4-MMC at 20 eV.....	250
Figure 7-13 DESI-MS spectra of A: 4-MMC, caffeine and paracetamol as a mixture; B: 4-MMC, caffeine, paracetamol and. codeine-D <sub>6</sub> as a mixture. ....	252
Figure 7-14 Structure of drug compounds used in selectivity study. ....	253
Figure 7-15 Ion suppression/enhancement present in different mixtures analysed using DESI-MS. ....	254
Figure 7-16 Calibration curve for 4-MMC in a mixture of caffeine and MA. ....	255
Figure 7-17 Molecular structure of methylone. ....	256
Figure 7-18 DESI-MS spectra of 4-MMC and methylone (with codeine-D <sub>6</sub> IS). ....	257
Figure 7-19 DESI-MS/MS spectra of A: methylone, B: 4-MMC at 20 eV.....	257
Figure 7-20 Proposed collision induced dissociation of the [M+H] <sup>+</sup> ion of methylone. ....	258
Figure 7-21 DESI-MS/MS spectra for A: 4-MMC, B: 4-DEAB, C: mixture of 4-MMC and 4-DEAB at 20 eV. ....	259
Figure 7-22 Proposed collision induced dissociation of the [M+H] <sup>+</sup> ion of 4-DEAB.....	259
Figure 7-23 DESI-MS/MS spectra for A: 1B3P, B: 4-MMC, C: mixture of 4-MMC and 1B3P at 20 eV. ....	260
Figure 7-24 Proposed collision induced dissociation of the [M+H] <sup>+</sup> ion of 1B3P. ....	260
Figure 7-25 DESI-MS spectra of 4-MMC. ....	264
Figure 8-1 A: DESI-MS/MS spectra of MA, B: DESI-MS/MS spectra of phentermine. ....	274

## List of tables

Table 3-1 List of mixtures tested for optimal solvent composition.....	78
Table 3-2 Design layout for 2 <sup>3</sup> design. ....	80
Table 3-3 Factor levels for drug analysis using DESI-MS.....	81
Table 3-4 Optimal parameters for caffeine. ....	91
Table 3-5 Comparison of the reproducibility of signal intensity of 4-MMC on three different plates, n=5.....	102
Table 3-6 Summary of optimised values for drug compounds; set values: d <sub>1</sub> =3 mm, d <sub>3</sub> =5 mm, β=15°, α=55°, solvent flow rate=0.21 mL/hr, high voltage=4 kV, gas flow rate=100 psi. ....	103
Table 4-1 Limit of detection of different ATS, n=3.....	127
Table 4-2 Mass accuracy of ATS, using positive ion mode.....	152
Table 4-3 Mass accuracy of MS/MS ions using positive ion mode at 20 eV.....	153
Table 4-4 Compounds detected in “Jack3d”, MDMA (tablet and synthesised base), and PMMA (synthesised base) using DESI-MS, GC-MS, and LC-MS. ....	154
Table 5-1 Limit of detection, intra-day and inter-day precision of cocaine, n=3.....	162
Table 5-2 Mass accuracy of cocaine and related compounds using positive ion mode.....	184
Table 5-3 Mass accuracy of MS/MS ions using positive ion mode at 20 eV.....	185
Table 5-4 Compounds detected in cocaine samples using DESI-MS, GC-MS, and LC-MS. ....	186
Table 6-1 Limit of detection of piperazine analogues, n=3. ....	199
Table 6-2 Mass accuracy of piperazine and related compounds using positive ion mode. ...	233
Table 6-3 Mass accuracy of MS/MS ions using positive ion mode at 20 eV.....	233

Table 6-4 Compounds detected in piperazine analogues using DESI-MS, GC-MS, and LC-MS. .....	235
Table 7-1 Statistical comparison of DESI-MS, GC-MS, and LC-MS in the analysis of 4-MMC.	240
Table 7-2 List of by-products found in synthesised 4-MMC samples with corresponding m/z values. ....	243
Table 7-3 Calculated concentrations of M1 using pure and mixed calibrator calibration curves. .....	256
Table 7-4 Comparison of codeine-D <sub>6</sub> and 4-MMC-D <sub>3</sub> , as internal standards. ....	262
Table 7-5 Optimising codeine-D <sub>6</sub> IS concentration, n=3. ....	262
Table 7-6 Purity of M1, M2, M3, M4 as determined using DESI-MS, GC-MS, and LC-MS. ....	263
Table 7-7 Mass accuracy of 4-MMC and related compounds using positive ion mode. ....	264
Table 7-8 Mass accuracy of MS/MS fragments using positive ion mode at 20 eV. ....	265
Table 7-9 Compounds detected in 4-MMC samples using DESI-MS, GC-MS and LC-MS. ....	266
Table 8-1 Results of Marquis reagent with BZP. ....	270
Table A-1 International seizures classified by drug type. ....	285

## Abbreviations

1B3P – 1-Benzyl-3-pyrrolidinol  
2C-I – 4-Iodo-2,5-dimethoxyphenethylamine  
4-DEAB – 4-(Diethylamino)benzaldehyde  
4-F-AP – 4-Fluoroamphetamine  
4-MMC – 4-Methylmethcathinone  
4-MTA – 4-Methylthioamphetamine  
4-OH-AP – 4-Hydroxyamphetamine  
5-HT – Serotonin  
ABDF – Bromobenzodifuranylisopropylamine  
AccuTOF – Accurate time-of-flight  
AF – Ammonium formate  
AFP – Australian Federal Police  
AGAL – Australian Government Analytical Laboratories  
AIDIP – Australian Illicit Drug Intelligence Program  
AP – Amphetamine  
API – Atmospheric pressure ionisation  
APPI – Atmospheric pressure photoionisation  
AP-MALDI – Atmospheric pressure – matrix assisted laser desorption ionisation  
ASAP – Atmospheric-pressure solids analysis probe  
ATR – Attenuated total reflection  
ATS – Amphetamine-type substances  
BE – Benzoyllecgonine  
BZP – *N*-Benzylpiperazine or 1-benzylpiperazine  
CC – Cinnamoyl cocaine  
CCD – Central composite design  
cps – Counts per second  
 $C_{\text{the}}$  – Theoretical concentration  
DAPPI – Desorption atmospheric pressure photoionisation  
DART – Direct analysis in real time  
DBZP – 1,4-Dibenzylpiperazine  
DESI – Desorption electrospray ionisation  
DMA – *N,N*-Dimethylamphetamine



DMAA – Dimethylamylamine  
 DMMP – Dimethyl methylphosphonate  
 DMS – Dimethyl sulfone  
 EBCP – Ethyl 1-benzyl-4-carboxypiperazine  
 EC – Ethyl centralite  
 EI – Electron ionisation  
 EIC – Extracted ion chromatogram  
 ELDI – Electrospray-assisted laser desorption ionisation  
 EME – Ecgonine methyl ester  
 EP – Ephedrine  
 ESI – Electrospray ionisation  
 FA – Formic acid  
 FFD – Full factorial design  
 FI – Flow injection  
 FTIR – Fourier transform infrared  
 GC-FID – Gas chromatography - flame ionisation detector  
 GC-MS – Gas chromatography - mass spectrometry  
 GSR – Gun-shot residue  
 H<sub>2</sub>O – Water  
 HCl – Hydrochloride  
 HPLC – High performance-liquid chromatography  
 HS-GC – Head space - gas chromatography  
 IRMS – Isotope ratio - mass spectrometry  
 IS – Internal standard  
 IT – Ion trap  
 KBr – Potassium bromide  
 K-tBuO – Potassium tert-butoxide  
 LC – Liquid chromatography  
 LC-MS – Liquid chromatography-mass spectrometry  
 LOD – Limit of detection  
 LOL – Limit of linearity  
 LOQ – Limit of quantitation  
 LSD – Lysergic acid diethylamide  
 M – Mean of the experimentally determined concentrations

MA – Methylamphetamine  
MALDESI – Matrix-assisted laser desorption electrospray ionisation  
MBCP – Methyl 1-benzyl-4-carboxypiperazine  
MC – Methyl centralite  
mCPP – 3-Chlorophenylpiperazine  
MDA – 3,4-Methylenedioxyamphetamine  
MDDMA – 3,4-Methylenedioxydimethylamphetamine  
MDEA – 3,4-Methylenedioxyethylamphetamine  
MDHOET – 3,4-Methylenedioxy-*N*-(2-hydroxyethyl)amphetamine  
MDMA – Methylenedioxymethylamphetamine ('Ecstasy')  
MDP-2-P – 3,4-Methylenedioxyphenyl-2-propanone  
MDP-2-POH – 3,4-Methylenedioxyphenyl-2-propanonol  
MeOH – Methanol  
MeOPP – 4-Methoxyphenylpiperazine  
MS – Mass spectrometry/Mass spectrometer  
MS/MS – Tandem mass spectrometry  
MUX – Multiplexed  
NHSP – National Heroin Signature Program  
NMI – National Measurement Institute  
NMR – Nuclear magnetic resonance  
OFAT – One-factor-at-a-time  
OMPP – 2-Methoxyphenylpiperazine  
P2P – Phenyl-2-propanone  
PCDL – Personal Compound Database and Library  
pEP – Pseudoephedrine  
PMA – 4-Methoxyamphetamine  
PMMA – 4-Methoxymethylamphetamine  
pMMA – Polymethyl methacrylate  
PMP-2-P – 4-Methoxyphenyl-2-propanone  
PN – Piperonyl nitrile  
PTFE – Polytetra fluoroethylene (Teflon)  
PVC – Polyvinyl chloride  
QC – Quality check samples  
QTOF – Quadrupole time-of-flight

IR – Infrared

RDX – Trinitrohexahydro-1,3,5-triazine

RE – Relative error

RSD – Relative standard deviation

SEM-EDX - Scanning electron microscopy – energy dispersive X-ray

SD – Standard Deviation

SNR – Signal-to-noise ratio

TFMPP – 3-Trifluoromethylphenylpiperazine

THC –  $\Delta^9$ -Tetrahydrocannabinol

TLC – Thin-layer chromatography

TOF – Time-of-flight

UNODC – United Nations Office on Drugs and Crime

US – United States

UV – Ultraviolet

UV-DAD – Ultraviolet diode array detector

## Abstract

Desorption electrospray ionisation - mass spectrometry (DESI-MS) is an ambient ionisation technique that can be applied to the analysis of illicit drugs and novel drug analogues in seized drug material. Currently used preliminary identification techniques lack sensitivity and selectivity and are prone to false positive and false negative results. Therefore, it was important to investigate the use of DESI-MS as a novel preliminary identification technique in the analysis of a range of compounds with the potential for future automated library matching aiding in the rapid identification of unknowns.

In this research, 4-methylmethcathinone (mephedrone or 4-MMC), cocaine, 1-benzylpiperazine (BZP), 3-trifluoromethylphenylpiperazine (TFMPP), 3-chlorophenylpiperazine (mCPP), 4-methoxyphenylpiperazine (MeOPP) and other amphetamine-type substances (ATS) such as amphetamine (AP), methylamphetamine (MA), 3,4-methylenedioxymethylamphetamine (MDMA), *N,N*-dimethylamphetamine (DMA), 4-methoxyamphetamine (PMA), and 4-methoxymethylamphetamine (PMMA) were the drugs of interest since they are increasingly prevalent drugs of abuse globally.

The optimisation of the technique and the application of DESI-MS to the analysis of these compounds were demonstrated. A particularly suitable surface, semi-porous polytetrafluoroethylene (PTFE, Teflon) was utilised, as it gave superior signal stability and reproducibility as compared to other surfaces (polymethyl methacrylate (pMMA) and polyvinyl chloride (PVC)).

The limits of detection (LOD) of 4-MMC and the piperazine analogues were determined to range between 0.0023 - 2.30  $\mu\text{g}/\text{mm}^2$ . The LOD of the ATS was determined to be in the range 0.02 - 2.80  $\mu\text{g}/\text{mm}^2$ . DESI-MS was also utilised in the preliminary analysis of illicit cocaine samples. The LOD of cocaine was determined to be 3.47  $\mu\text{g}/\text{mm}^2$ . The chemical profiles obtained using DESI-MS were also compared to two current confirmatory analysis techniques, gas chromatography – mass spectrometry (GC-MS) and liquid chromatography – mass spectrometry (LC-MS). The by-products and impurities detected were used to link samples to their synthetic route of manufacture and to the precursors used. The detection of truxillines in the seized cocaine samples aided in determining their geographical origin.

Selectivity and matrix effects were evaluated for the compounds of interest in each study. The effect of adulterants including caffeine, procaine, levamisole, lignocaine, paracetamol, and atropine on the detectability of cocaine were investigated. The detectability of ATS were evaluated by adding caffeine, paracetamol, magnesium stearate, and dimethyl sulfone. Piperazine compounds were adulterated using caffeine and a mixture of piperazines (TFMPP and BZP) was also evaluated since these are commonly found in combination. 4-MMC was adulterated with caffeine, paracetamol, MA, phentermine, AP, MDMA, 4-hydroxyamphetamine, 4-fluoroamphetamine, nordiazepam, diazepam, oxazepam, cocaine, heroin, methadone, cathine, cathinone, 4-diethylaminobenzaldehyde, 1-benzyl-3-pyrrolidinol, and methylone. In most cases, despite the presence of ion enhancement or suppression due to the addition of adulterant, the analytes remained detectable.

Quantitative experiments for 2  $\mu$ L spotted solutions of 4-MMC, using codeine-D<sub>6</sub> as an internal standard, introduced in the desorption spray solvent, showed a linear correlation ( $R^2 > 0.9840$ ) over the range 50 – 800  $\mu$ g/mL. The precision for triplicates analysed on five different days ( $n = 15$ ) was <38 % RSD. The accuracy, expressed as relative error, was <13 %. Identification based on accurate mass and MS/MS spectra aided in discriminating compounds with the same molecular formula. The results obtained using DESI-MS demonstrate its applicability in the rapid qualitative analysis (and preliminary quantitative analysis of 4-MMC) of cocaine, 4-MMC, BZP, TFMPP, mCPP, MeOPP and ATS.